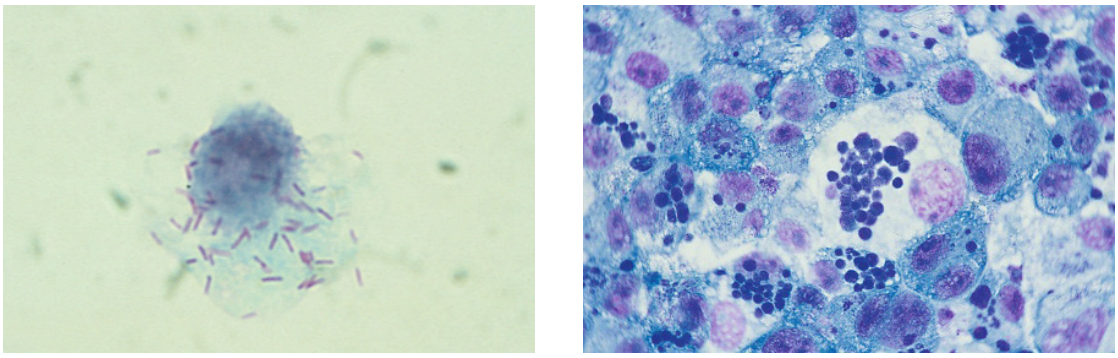
GNR: Intracellular Obligate Parasites

Introduction

This lesson explores a set of **obligate intracellular bacteria.** That is, they must live within the cytoplasm of another cell. To fit into another cell, replicate, and grow necessitates that these organisms be relatively very small. The two major bacteria discussed in this lesson, *Chlamydia* and *Rickettsia*, are also **energy parasites**: being too small to produce their own ATP, they must use that made by the host cell. *Chlamydia* species infect with elementary bodies, and can exist outside cells. *Rickettsia* species require an arthropod vector to be transmitted.

We discuss *Chlamydia* species first, including the species that causes the sexually transmitted infection called chlamydia. We then continue with *Rickettsia* species, including the species that causes Rocky Mountain spotted fever and typhus. We close the lesson with *Rickettsia*-like organisms; *Ehrlichia*, *Anaplasma*, and *Coxiella*. Definitely learn *Chlamydia*, probably learn *Rickettsia*, and the others are only for augmentation. All the organisms discussed in this lesson have **Gram-negative physiology**—outer plasma membrane, small cell wall, LPS—but are intracellular, so don't stain well. Specialized immunofluorescence or Giemsa staining can reveal them. However, the objective is less about identification and more about mastering the diseases they cause, and knowing that a negative Gram stain on an obvious bacterial infection is likely to be an intracellular pathogen.



(a) (b)

Figure 12.1: Obligate Intracellular Bacteria
(a) The purple elongated shapes (Gram-negative rods) can be seen against the backdrop of a nucleus, all enclosed by a wispy, near-see-through cytoplasm. (b) The large homogeneous structures surrounded by balls of light blue are leukocytes. The conglomeration of small intensely purple dots surrounded by white is the start of a morula of Ehrlichiosis.

Chlamydia

Chlamydia species are Gram-negative rods, but because they are **so small** and must live **within other cells**, they will effectively **never be seen on Gram stain**. They go back and forth between reticulate bodies and elementary bodies. **Elementary bodies** (EBs) are the metabolically inactive, nonreproducing form that is also the infective form. It induces endocytosis and is brought into the cell within a phagosome. EB outer membrane proteins prohibit the fusion of the lysosome with the phagosome. The phagosome then becomes a cozy place to replicate. Elementary bodies then **reorganize** into reticulate bodies inside the phagosome. The **reticulate bodies** (RBs) are the metabolically active form while within infected cells, replicating by binary fission, producing more reticulate bodies, who then reorganize back into elementary bodies. When a critical mass is reached, the cell lyses. Elementary bodies Enter the cell, reticulate bodies replicate in the cell. *Chlamydia* was once thought to be a virus, its size so small and impact on the cell similar to a lytic phage.

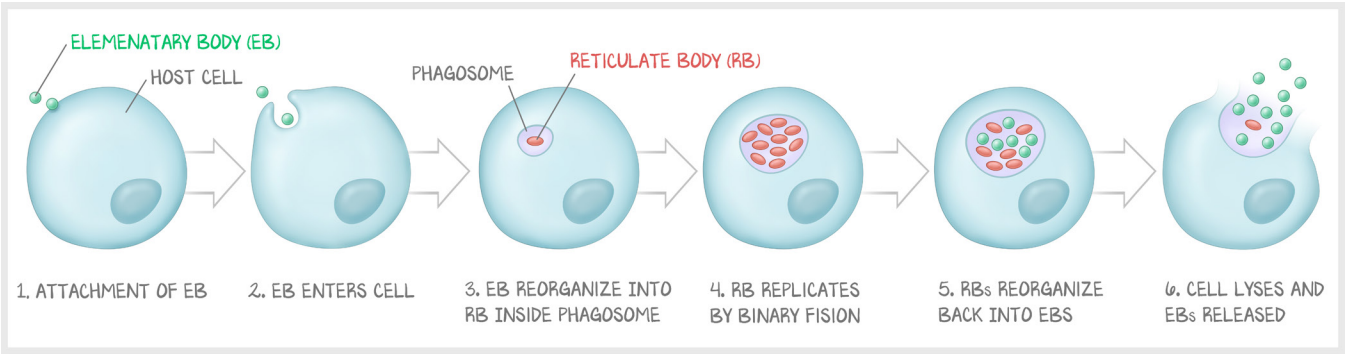


Figure 12.2: *Chlamydia* Life Cycle
Chlamydia is an obligate intracellular parasite, yet it is able to exist outside of a cell. In its hardy elementary body (EB) form, the tiny bacterium (1) attaches to the cell surface and (2) induces clathrin-mediated endocytosis to enter the cell. Once within the endosome, the bacterium prevents fusion with lysosomes, using the endosome as a membrane-bound, protected area replicate. To do that, the metabolically inactive elementary body (3) reorganizes into a reticulate body (RB). The RB is the metabolically active, reproducing form, stealing energy and resources from the host cell to (4) replicate by binary fission. As the reticulate bodies amass a critical number, they (5) reorganize back into elementary bodies before (6) inducing cell lysis and releasing EBs to infect other cells. For a time, *Chlamydia* was thought to be a lytic virus.

Chlamydia trachomatis ("*Chlamydia*")

Chlamydia is a ubiquitous bacterium that causes lots of problems for the world. What type of disease it causes is dependent on its membrane proteins. *Chlamydia* is divided into serovars based on those proteins. There are three syndromes that *Chlamydia* causes. Each syndrome is caused by specific serovars. Serovars **A, B,** and **C** are responsible for African Blindness in Children. A, B, and C cause the disease named **trachoma.** Serovars **L1, L2,** and **L3** cause the disease Lymphogranuloma venereum (LGV). Serovars **D–K** (all the remaining serovars) cause the disease chlamydia (a **urogenital infection**), and are the serovars/disease type we see in the United States. *Chlamydia* can infect any nonciliated mucosa such as the GI tract, vagina, mouth, anus, urethra, and even the conjunctiva of the eye. Its elementary bodies are hardy enough to survive fecal-oral transmission, or hand-to-eye transmission.

SEROVAR	LOCATION	DISEASE
A, B, C	Africa, Third World	Trachoma, blindness, African Blindness in Children
D–K	United States	Urogenital infections, cervicitis, PID, urethritis with discharge
L1, L2, L3	Everywhere	Lymphogranuloma venereum, granulomas in the lymph nodes

Table 12.1: *Chlamydia* Diseases by Serovar

Trachoma is a chronic disease caused by serovars A–C. It is the **eye disease** variant of *Chlamydia*. As a child, the patient is colonized with *Chlamydia*. Poor hand hygiene inoculates the bacteria into the eyelid. The first time it causes a problem is an acute **follicular conjunctivitis.** We call that a sty. No big deal. Recurrent follicular conjunctivitis causes a scarring of the eyelid on the conjunctival side. Repeated scarring causes the eyelashes to turn inward. The turned-in eyelashes abrade the cornea, eventually resulting in corneal ulceration, scarring, and obstructive pannus formation. Trachoma is the world's **leading cause of preventable blindness**. Because many children have colonized their respiratory or gastrointestinal tracts, they serve as the reservoir for disease. Trachoma blindness occurs because a child infects another child's eyes. Without treatment, the progression to trachoma blindness starts. One dose of **azithromycin** can clear the patient of *Chlamydia*, preventing trachoma from happening. Good **hand-**

face hygiene could go a long way. Unfortunately, trachoma is most prevalent in populations without resources, antibiotics, or the ability for hand hygiene.

Urogenital chlamydia is caused by serovars **D–K**. Chlamydia ("The Clap") infects the urethra, cervix, and fallopian tubes. It causes a thick white or yellow discharge from the cervix (women are often asymptomatic) or penis. Whatever you learned for gonorrhea, learn for chlamydia. In fact, if either is found, both are treated. In the US, chlamydia is a major cause of **infertility**, and is the most commonly sexually transmitted bacterial disease. Treatment is with azithromycin once or doxycycline for a week. In the US, urine is tested with **nucleic acid amplification tests** (PCR) to make the diagnosis. Pregnant women with urogenital chlamydia can infect their newborn during delivery, resulting in a **neonatal conjunctivitis** (normal eyes in the first week, then bilateral purulent eyes in the second week). Those neonates with neonatal conjunctivitis have been exposed to chlamydia, and therefore are at higher risk of developing **neonatal pneumonia** (characterized by a staccato cough three weeks after birth).

Lymphogranuloma venereum (LGV) is caused by serotypes **L1, L2, L3**. It is a form of sexually transmitted disease with escalation beyond the urethra or cervix. Initially, the primary lesion—a **painless ulcer**—develops. Subsequently, and usually after the primary lesion heals spontaneously, **inguinal** lymph nodes swell. **Granulomas** form to fight the intracellular pathogen. The lymph nodes rupture, creating draining tracts and ulcerations. This is the *Chlamydia* in the nodes. Left untreated, the lymph nodes become swollen and firm, blocking lymph drainage from the leg, leading to fistulas, ulcers, and genital elephantiasis.

Chlamydia pneumoniae ("Atypical Pneumonia")

C. pneumoniae causes an atypical pneumonia worldwide. Atypical pneumonias are not typically virulent or dangerous. They cause a dry cough, sometimes a fever, but rarely hypoxemia or shock. Because they are diagnosed as "ambulatory pneumonias," they are treated with azithromycin and are rarely diagnosed further. Associated with acceleration of atherosclerosis.

Chlamydia psittaci ("Parrot Pneumonia")

C. psittaci causes the same atypical pneumonia picture as above, only the vector is in feathers and feces of birds. **Parrots** get all the blame, though over 100 species of birds can carry the bacterium. Veterinarians, poultry shops, and carrier-pigeon attendants are at highest risk. Psittacosis is a viral-appearing syndrome that self-resolves.

Rickettsia

Rickettsia species are obligate intracellular organisms that live within the **cytoplasm** of eukaryotic cells. Unlike the other obligate intracellular bacteria in this lesson, after endocytosis occurs, rather than preventing fusion to the lysosome, *Rickettsia* lyse the phagosome and live in the cytoplasm. They have Gram-negative physiology—outer plasma membrane with LPS and a peptidoglycan cell wall. However, the peptidoglycan cell wall is so small, and the bacteria in the cytoplasm of another cell, they rarely are capable of taking up a Gram stain. In addition, their LPS is so poorly antigenic, the endotoxin activity is minimal.

The most important *Rickettsia* to know is *Rickettsia rickettsii*, the one that causes **Rocky Mountain spotted fever** (RMSF). It is the prototype for the spotted fever group of *Rickettsia*. When we use "Rickettsia" by itself (without italics), we mean RMSF. We will not mention the others in that group. Lower-yield *Rickettsia* are those of the **typhus group** *Rickettsia prowazekii* and *Rickettsia typhi*. We will not refer to these as *Rickettsia*, but simply as *prowazekii* and *typhi*.

Typhus fever is not Typhoid Mary. Mary was typhoid, typhus-like. Typhus describes the syndrome of fever, headache, and a rash. Not very specific. RMSF has headache, fever, and a centripetal rash, so is not typhus. Typhus fever has headache, fever, and a noncentripetal rash, so is typhus. Don't let the word typhus trip you up.

Rickettsia rickettsii ("Rocky Mountain Spotted Fever")

Rocky Mountain spotted fever is most prevalent in the **Appalachian Mountains** and NOT the Rocky Mountains (thanks, nomenclature). The disease is most commonly seen in the Carolinas and Tennessee. We suggest you learn RMSF, and think of the R as being *Rickettsia* instead of Rocky. It is caused by *Rickettsia rickettsii*, a bacterium carried in **ticks**. Ticks are the vector, *Rickettsia rickettsii* is the infectious agent. The dermacentor species of tick requires **prolonged feeding times** to infect with *Rickettsia* (> 6 hours), and therefore early detection and removal of the tick prevents infection with *Rickettsia*. If transmitted, *Rickettsia* gets into the bloodstream and disseminates. Bacteria in the blood the body generally tends to notice. **Fever and a headache** predominate in the syndrome, which is also associated with toxic symptoms such as myalgias, nausea, vomiting, etc. Rickettsia then replicates within the endothelial cells—the cells lining the blood vessels. The localized inflammation of the blood vessels (vascular-itis) is the definition of **vasculitis.** Small-vessel vasculitis results in microthrombi and hemorrhage. It is this hemorrhage and inflammation that cause the characteristic rash and contribute to organ failure.

The patient is exposed and bitten by a tick. No one knows. Seven days later, fever and a rash develop. For two days, the only symptoms are fever, headache, myalgias, nauseas, etc.—vague infection symptoms. Then the rash starts on day 9.

The disease is most characterized by a **centripetal rash** (starts on wrists and ankles, then spreads back towards trunk and outwards to the arms) that **also involves the palms and soles.** Because blood vessels are in every organ, any organ can be affected. Inflammation causes vascular permeability and extravasation of plasma, leading to poor perfusion of every affected organ.

TREPONEMA PALLIDUM	*RICKETTSIA RICKETTSII*	COXSACKIE A VIRUS
Syphilis	RMSF	Hand-foot-mouth disease
Diffuse macular rash everywhere at once . . .	Wrists and ankles, then ascending to trunk . . .	Lesions only on the mouth . . .
And palms and soles	And palms and soles	And palms and soles

Table 12.2: Infectious Causes of Rashes on the Palms and Soles

Laboratory confirmation is challenging. Being intracellular, the organisms do not stain well. Giemsa stain or radiolabeled fluoroscopy can be implemented, but may not be rapidly available—reference labs (send outs) are the only ones who can confirm it. Same with cultures—easy to do if you have a specialized laboratory. The Weil-Felix test is no longer recommended. So if you can't diagnose it right away, what are you to do? If symptoms are present and the patient is in or recently came from an endemic area, **start doxycycline** before the diagnosis is certain. This is true even in children less than 8 years old (in whom tetracycline antibiotics are generally contraindicated).

The empiric treatment for meningitis includes ceftriaxone and vancomycin, neither of which works on *Rickettsia*. Meningitis? RMSF presents with fever and headache (which may prompt the treatment of meningitis with vancomycin and ceftriaxone) that precedes the rash. Therefore, rather than recognize this as rickettsial disease, clinicians may anchor on the meningitis diagnosis and then attribute the eruption of the rash to a medication side effect. RMSF is the "obvious meningitis" that isn't meningitis (lumbar puncture does not demonstrate thousands of neutrophils), and then has the ascending rash. The headache and the rash are from the vasculitis, not acute bacterial infection of the brain. The earlier doxycycline is started, the better the outcome.

Rickettsia prowazekii ("Epidemic Typhus")

Prowazekii causes **epidemic typhus**. An epidemic is the sudden onset and rapid spread of an infection across a population. Epidemic typhus is carried by **human lice**. It is the abundance of lice and close proximity of humans that causes the epidemic, and thus it is most likely to occur where humans congregate in large number—cities. The patient will have **fever and a headache** two weeks after exposure. After the fever has been present, the rash eventually starts (like Rickettsia). But the rash is not the centripetal ascending rash, but is **small pink macules** that **start on the trunk** and spread to the extremities **but spare the palms and soles**. *Prowazekii* does the same thing as *Rickettsia* did—gets into endothelial cells. Only *prowazekii* does it to larger vessels, which can result in **arterial thrombosis and gangrene**. Sporadic cases continue in the United States, occurring only because of the **flying squirrel vector**. A mention of flying squirrels implies epidemic typhus. You are unlikely to see a question that specifically calls out flying squirrels—it is too obvious an answer. Doxycycline is the treatment.

Rickettsia typhi ("Endemic Typhus")

Typhi causes **endemic typhus**. An endemic is an infectious disease that is highly prevalent and exists constantly in a population. Endemic typhus is carried by **rats** and transmitted to humans by **rat fleas**. The presentation of endemic typhus is identical but less severe and more insidious than epidemic typhus. Think of an epidemic as a sudden explosion of cases all at once, and endemic as a slow, undulating number of cases. Epidemic explodes; epidemic typhus causes high fevers of rapid onset. Endemic undulates; endemic typhus causes moderate fevers of insidious onset. The treatment and management are the same. Clean sanitation and pest eradication can avoid illness. Doxycycline is the treatment.

270+ *Ehrlichia* and *Anaplasma* ("Ehrlichiosis")

The microbiology between *Ehrlichia*/*Anaplasma* and *Rickettsia* is very different, but the presentation and disease are nearly the same. Ehrlichiosis presents as RMSF, but the rash isn't centripetal. It is carried in a tick vector; one week after tick exposure there is a **fever and a headache** followed by a **rash** (50% of the time). It is treated with doxycycline. Mortality is low (2–3%) if untreated, but even those that are treated tend to have a prolonged recovery time.

Ehrlichia/*Anaplasma* **remain in phagosome** and do **not fuse** with lysosome. Thus, replication can occur within the phagosome. **Elementary bodies** are the replicating form of the bacteria. A few days after infection, the elementary bodies assemble a **morula**, a membrane-bound (the original phagosome) mass. These are not only obligate intracellular bacteria, but have specific tropism for one type of cell. *Ehrlichia* invades **monocytes** (circulating macrophages), while *Anaplasma* invades **granulocytes** (neutrophils). Both impair phagocytes.

Since 1986, there have been 2,600 cases of *Ehrlichia* or *Anaplasma*. Yes, you are still expected to learn them.

Coxiella burnetii ("Q Fever")

Coxiella burnetii is also an **obligate intracellular** organism, but it is unique in that it **forms spores**. Because of the spores, it is **resistant to heat and drying**. And because it has a durable spore form, it can **survive outside a cell** and therefore also **requires no arthropod vector**. Spores live on dried placental material from sheep, goats, and cows and can infect soil. *Coxiella* is found in unpasteurized milk. *Coxiella* can cause an atypical pneumonia without rash, opposed to the other organisms in this lesson that presented with fever, headache ("meningitis") and a rash. It is a more chronic, indolent, gradual-onset fever. While this pneumonia is relatively mild, **Q fever** can result in endocarditis and granulomatous hepatitis, which can be fatal. Ticks do not transmit *Coxiella* to humans. Treatment is doxycycline.